AMENDMENT

In the Title

Please replace the title with the following title:

E. COLI AND *STREPTOMYCES* HOST CELLS THAT SYNTHESIZE METHYLMALONYL COA

In the Specification

Please replace the first paragraph on page 1 with the following paragraph:

C1

This application claims priority under 35 U.S.C. § 119(e) to application Serial No. 60/159,090 filed 13 October 1999; Serial No. 60/206,082 filed 18 May 2000; and Serial No. 60/232,379 filed 14 September 2000, which are expressly incorporated herein by reference.

Please replace the paragraph on page 2, commencing at line 21, with the following paragraph:

C2

Additional problems that may need to be surmounted in effecting the production of polyketides in procaryotic hosts, especially those which do not natively produce polyketides, include the presence of enzymes which catabolize the required starter and/or extender units, such as the enzymes encoded by the *prp* operon of *E. coli*, which are responsible for catabolism of exogenous propionate as a carbon and energy source in this organism. In order to optimize production of a polyketide which utilizes propionyl CoA as a starter unit and/or utilizes its carboxylation product, methylmalonyl CoA as an extender unit, this operon should be disabled, except for that portion (the E locus) which encodes a propionyl CoA synthetase. Any additional loci which encode catabolizing enzymes for starter or extender units are also advantageously disabled.

Please replace the paragraph on page 4, commencing at line 11, with the following paragraph:

In the illustrative example below, *E. coli* is modified to effect the production of 6-dEB, the polyketide precursor of erythromycin. The three proteins required for this synthesis, DEBS1, DEBS2 and DEBS3 are known and the genes encoding them have been cloned and sequenced. However, a multiplicity of additional PKS genes have been cloned and sequenced as well, including those encoding enzymes which produce the polyketide precursors of avermectin, oleandomycin, epothilone, megalomycin, picromycin, FK506, FK520, rapamycin, tylosin, spinosyl, and many others. In addition, methods to modify native PKS genes so as to alter the nature of the polyketide produced have been described. Production of hybrid modular PKS proteins and synthesis systems is described and claimed in U.S. patent 5,962,290. Methods to modify PKS enzymes so as to permit efficient incorporation of diketides is described in U.S. patent 6,080,555. Methods to modify PKS enzymes by mixing and matching individual domains or groups of domains is described in U.S. Serial No. 09/073,538. Methods to alter the specificity of modules of modular PKS's to incorporate particular starter or extender units are described in U.S. Patent No. 6,221,641. Improved methods to prepare diketides for incorporation into polyketides is described in U.S. Serial No. 09/492,733. Methods to mediate the synthesis of the polyketide chain between modules are described in U.S. Serial No. 09/500,747. The contents of the foregoing patents and patent applications are incorporated herein by reference.

C3

Please replace the paragraph on page 11, commencing at line 10, with the following paragraph:

For either *in vivo* or *in vitro* production of the polyketides, acyl transferase domains with desired specificities can be incorporated into the relevant PKS. Methods for assuring appropriate specificity of the AT domains is described in detail in U.S. Patent No. 6,221,641, the contents of which are incorporated herein by reference, to describe how such domains of desired specificity can be created and employed. Also relevant to the use of these enzymes *in vitro* or the genes *in vivo* are methods to mediate polyketide synthase module effectiveness by assuring appropriate transfer of the growing polyketide chain from one module to the next. Such methods are

C4

C4 described in detail in U.S. Serial No. 09/500,747 filed 9 February 2000, the contents of which are incorporated herein by reference for this description.

Please replace the paragraph on page 14, commencing at line 27, with the following paragraph:

C5 One useful approach is to modify the KS activity in module 1 which results in the ability to incorporate alternative starter units as well as module 1 extended units. This approach was illustrated in PCT application US/96/11317, incorporated herein by reference, wherein the KS-1 activity was inactivated through mutation. Polyketide synthesis is then initiated by feeding chemically synthesized analogs of module 1 diketide products. The methods of the invention can then be used to provide enhanced amount of extender units.

Please replace the paragraph on page 17, commencing at line 18, with the following paragraph:

C6 As disclosed in U.S. Patent No. 6,033,883, incorporated herein by reference, a wide variety of hosts can be used, even though some hosts natively do not contain the appropriate post-translational mechanisms to activate the acyl carrier proteins of the synthases. These hosts can be modified with the appropriate recombinant enzymes to effect these modifications.

Please replace the paragraph on page 22, commencing at line 21, with the following paragraph:

C7 Plasmids pRSG32 (DEBS1+TE) and p132 (a plasmid containing the α and β components of propionyl-CoA carboxylase) were cotransfected into BAP1. Cultures of 10 ml M9 minimal media were grown to mid-log phase levels and concentrated to 1 ml for induction with IPTG and the addition of 0.267 mM ^{14}C-propionate. The samples were then incubated at 22°C for 12-15 hours. The culture supernatant was then extracted with ethyl acetate for analytical TLC. A product ran with the expected positive control and this same product was undetectable when using either wild type BL-21 (DE3) or removing p132. Thus, the carboxylase forms the correct stereoisomer.